PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Frederick M. BOYCE
Serial No.: 08/752,032
Filed : November 19, 1996
Title : USE OF A BACULOVIRUS TO EXPRESS AN EXOGENOUS GENE IN A MAMMALIAN CELL

Art Unit: 1819
Examiner: B. Campbell

Box AF

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF DR. JAMES BARSOUM UNDER 37 CFR §1.132

I, James Barsoum, declare:

1. I am a Senior Scientist at Biogen, Inc., the licensee of the subject matter described and claimed in USSN 08/752,032, captioned above (hereinafter "the '032 application").

2. My colleagues and I conducted the experiments described below, which show that a baculovirus can be used as described in the '032 application to express an exogenous gene in a mammal and obtain a significant therapeutic effect. Using both *in vivo* and *ex vivo* gene delivery methods, we showed that baculovirus-mediated expression of human interferon-β (hIFNβ) in two mouse models of human cancer leads to a significant and sustained amelioration of disease.

Experiment 1: *In Vivo* Gene Delivery

3. To produce the baculovirus used in this experiment, a nucleic acid encoding hIFNβ was cloned into a baculovirus

termed CMV-BV. In this baculovirus, expression of the exogenous gene was controlled by a cytomegalovirus (CMV) immediate early promoter (as described at page 18, lines 2-5, of the specification). The baculovirus also contained an SV40 polyadenylation site downstream of the exogenous gene (as described at page 7, lines 7-10). The virus was propagated and purified by sucrose gradient ultracentrifugation (as described in the specification at page 8, lines 22-26). The final purified virus stock had a titer of approximately 1 x 10^{10} plaque forming units/ml (pfu/ml).

4. This experiment used an art-accepted mouse model of human colon cancer. Human colon carcinoma cells, termed KM12L4A cells, were grown in 150 mm tissue culture dishes in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and 4 mM glutamine. Approximately 2 x 10^6 cells were injected subcutaneously into a flank of each of four immune-deficient mice (Balb/c nu/nu mice). The injected carcinoma cells rapidly developed into tumors that were 9-12 mm in diameter.

5. Approximately 1 x 10^9 pfu of the baculovirus expressing hIFNβ then were injected into two of the tumor-bearing mice. The other two mice received approximately 1 x 10^9 pfu of a control virus, CMVZ-BV, which contains a β-galactosidase reporter gene rather than an hIFNβ gene. In each case, the virus was injected, using a 28 gauge syringe, into the approximate center of the tumor in a volume of 0.1 ml.

6. Tumors in the mice that received the control virus continued to grow after injection of the virus. These tumors

quickly reached approximately 10% of the total body weight of the mouse, at which point the mice had to be sacrificed in compliance with animal guidelines. In contrast, tumors in the mice that received the baculovirus expressing hIFNβ showed signs of regression at just one day after injection of the baculovirus. The tumors in these mice completely regressed within 7 days of injecting the baculovirus expressing the exogenous hIFNβ gene. Scar tissue at the site of injection subsequently healed completely. These mice remain tumor free to date, which is approximately 3 months after conclusion of the experiment. In conclusion, this experiment shows that a baculovirus expressing an exogenous gene can provide a significant and sustained therapeutic effect *in vivo*.

Experiment 2: *Ex Vivo* Gene Delivery

7. In this experiment, *ex vivo* gene delivery methods were used to deliver a baculovirus carrying an exogenous hIFNβ gene into cancer cells. The cancer cells subsequently were implanted into animals, and a sustained, therapeutic effect was obtained due to expression of the exogenous hIFNβ gene *in vivo*.

8. The CMV-BV baculovirus carrying the hIFNβ coding sequence, as described above, was used in this experiment. Human breast cancer cells, MDA-MB-468 cells, were grown in 150 mm tissue culture dishes in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and 4 mM glutamine. The baculovirus carrying the exogenous gene was added to the culture

medium at 200 pfu/cell, and the cells and virus were incubated overnight at 37°C in a CO_2 incubator.

9. MDA-MB-468 cells that were not infected with the baculovirus were used as a control. The uninfected and baculovirus-infected MDA-MB-468 cells were harvested with trypsin and counted. Approximately 2 x 10^6 baculovirus-infected cells were injected subcutaneously into a flank of each of four Balb/c nu/nu mice. Four other Balb/c nu/nu mice each received, by injection into a flank, approximately 2 x 10^6 uninfected cells. For the injections, the cells were contained in 0.1 ml of phosphate-buffered saline.

10. As shown in Table 1, tumors quickly developed in the mice that received the uninfected (i.e., untreated) cancer cells. The tumors in these control mice increased in size throughout the course of the six week experiment. On average, the tumors resulting from injection of the uninfected cells grew to 5.2 mm in diameter (at 7 days after infection) and increased in size to 7.6 mm (at the end of 6 weeks after injection).

Table 1: Tumor Size (mm)

Cell Treatment	**Week 1***	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Uninfected	5.2±0.1	5.6±0.1	5.9±0.1	6.1±0.3	6.8±0.3	7.6±0.5
BV-CMV-IFNβ Treated	1.9±1.3	0.5±1.0	0.6±1.0	0.5±1.0	0.6±1.3	0.7±1.4

* The table shows the average tumor size (from 4 mice) at the end of each week following injection.

11. In contrast to the results obtained with mice that received uninfected cancer cells, a significant therapeutic effect was observed with the mice that received cancer cells which were treated with baculovirus expressing hIFNβ. At week 1 after infection, tumors in the mice that received baculovirus-infected cells were significantly smaller (1.9 mm) than were the tumors in the mice that received uninfected cells (5.2 mm). Thus, tumor formation was significantly inhibited in the mice that received baculovirus-infected cancer cells, relative to formation of tumors in the mice that received uninfected cancer cells.

12. The therapeutic effect of expressing hIFNβ from baculovirus was sustained over time. As shown in Table 1, the average tumor in the mice that received the baculovirus-infected cells was 1.9 mm in diameter after one week and regressed to 0.7 mm by the end of the six-week experiment. In fact, by week 6, the tumors in 3 of the 4 baculovirus-infected mice had regressed completely. This decrease in the size of tumors in the mice that received cancer cells infected with baculovirus expressing hIFNβ is in direct contrast to the observed increase in the size of tumors in mice that received uninfected cancer cells. These data indicate that the therapeutic effect of the baculovirus expressing the exogenous hIFNβ gene was sustained over at least a six-week time period and suggest that continued expression of IFNβ led to the death of most tumor cells.

Summary

13. To conduct the experiments described above, we used art-accepted animal models of human colon and breast cancers and showed that baculovirus-mediated gene expression *in vivo* provides a significant therapeutic effect that is sustained over time. A significant therapeutic effect was obtained with both *in vivo* and *ex vivo* methods of delivering the baculovirus and exogenous gene to cells of the treated animal.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: June 15, 1998

James Barsoum, Ph.D.

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